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Hypertonic sodium chloride induction of cyclooxygenase-2 occurs independently of NF-κB and is inhibited by the glucocorticoid receptor in A549 cells

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Abstract Cellular response to a hypertonic environment is important for fluid clearance in the lung. Hypertonicity modulates prostaglandin synthesis by influencing cyclooxygenase-2 (COX-2) expression in tissues such as liver and kidney via a mitogen-activated protein kinase (MAPK)-dependent pathway. However, little is known about COX-2 expression in response to hypertonicity in the lung. COX-2 mRNA accumulation induced by hypertonic NaCl was detected after 1 h of treatment, and COX-2 mRNA continued to accumulate until 18 h, the longest time point examined, in human alveolar epithelial A549 cells. This induction was a transcriptional event that occurred in the absence of the protein synthesis inhibitor cycloheximide and was the result of enhanced promoter activity, as examined with the use of full-length COX-2 promoter-driven reporter plasmids. The induction of COX-2 expression by hypertonic NaCl did not require the activation of NF-κB. The p38 MAPK inhibitor, SB203580, or MEK1/2 inhibitor, U0126, inhibited hypertonic induction of COX-2 expression. We examined whether the hypertonic induction of COX-2 was under the influence of glucocorticoid; we found that COX-2 promoter activity and mRNA and protein levels were depressed by dexamethasone and antagonized by the glucocorticoid receptor (GR) antagonist RU486. Our data demonstrate that the induction of COX-2 expression by hypertonic NaCl occurs independently of NF-κB and is inhibited by the GR in A549 cells.
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Keywords: COX-2; Hypertonic sodium chloride; NF-κB; Glucocorticoid receptor; Alveolar epithelial cells

1. Introduction

Salt and water transport play an important role in alveolar fluid clearance (AFC), and active sodium ion transport drives osmotic water transport in the lung. The alveoli must remain open and free from fluid for efficient gas exchange to occur [1]. Intact AFC, therefore, is critical in clearing fluid from the lungs at birth and keeping the alveolar space relatively fluid-free for adequate gas exchange under physiological conditions [2–4].

One enzyme that may be involved in this process is cyclooxygenase (COX), a key regulatory enzyme in the biosynthesis of prostaglandins (PGs) from arachidonic acid [5]. COX-2 has a diverse assortment of biological functions in mammalian tissues, such as regulation of vascular tone, expression and secretion of rennin, and salt and water homeostasis in the kidneys [6]. Recent studies on the effects of salt on COX-2 expression in the kidney have identified some mechanisms for the regulation of COX-2 expression. It was reported that a low-salt medium regulates COX-2 expression by p38- and NF-κB-dependent signaling pathways in cultured cortical cells from the thick ascending limb of the loop of Henle [6,7]. Hypertonic NaCl activated COX-2 in renal medullary interstitial cells through the transactivation of the epidermal growth factor receptor [8]. Pathways involving the transcription factor NF-κB and mitogen-activated protein kinase (MAPK) play a central role in the high-salt-mediated regulation of COX-2 expression in mammalian kidney cells [9,10]. All three members of the MAPK family (ERK, JNK-2, and p38) as well as Src kinases are required for tonicity-stimulated COX-2 expression in inner medullary collecting duct cells [10].

Cellular dysfunction induced by hypertonic NaCl in alveolar epithelial cells could play an important role in the lung fluid balance under both normal and pathological conditions. Saline infusion has been reported to have a significant influence on inflammation-related gene expression in the lung [11]. Previous studies have shown that the inhibition of prostaglandin synthesis inhibits the flow of liquid from the fetal lungs [12–14]. However, the effects of hypertonic stress on COX-2 expression in alveolar epithelial cells and the mechanism involved are not known. In the present study, we examined the hypertonic NaCl induction of COX-2 expression in the human alveolar epithelial cell line A549.

2. Materials and methods

2.1. Materials

Cycloheximide, pyrrolidine dithiocarbamate (PDTC), SB203580, U0126, urea, NaCl, mannitol, 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1), aldosterone, spironolactone, dexamethasone, RU486, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 were purchased from Sigma (St. Louis, MO, USA). Fetal calf serum (FCS), Trizol Reagent, and penicillin/streptomycin were purchased from GIBCO Invitrogen (Grand Island, NY, USA).

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2.2. Cell culture, transfection, and luciferase assays

A549 cells, a human pulmonary epithelial cell line, were grown in DMEM/Ham's F12 nutrient mixture containing 10% FCS and penicillin/streptomycin in a humidified 37 °C incubator. COX-2-Luc, a firefly luciferase reporter construct containing the mouse COX-2 gene promoter fragment (3.4 kb), −327/+59, a firefly luciferase reporter deletion construct of the human COX-2 promoter, KBM, an NF-κB binding region site-specific mutant of −327/+59, hα ENaC-Luc, a firefly luciferase reporter construct containing the human α ENaC promoter fragment (1.4 kb), and 3 × (NF-κB)tk-Luc, a firefly luciferase reporter construct containing three repeated NF-κB-responsive elements, were kindly provided Dr. Huifang Cheng (Vanderbilt University School of Medicine, Tennessee, USA) [6], Dr. Hiroyasu Inoue (Nara Women's University, Nara, Japan) [15], Dr. Christie P. Thomas (University of Iowa College of Medicine and the Veterans Affairs Medical Center, Iowa, USA) [16], and Dr. Sam Okret (Karolinska University Hospital Huddinge, Huddinge, Sweden) [17], respectively. A549 cells were transiently transfected with COX-2-Luc, −327/+59, KBM, hα ENaC-Luc, or 3 × (NF-κB)tk-Luc by electroporation. Electroporation was performed with a Gene Pulser II (Bio-Rad, Hercules, CA, USA). Cells were trypsinized, washed in cold PBS, and resuspended in PBS. A 400 μl portion of the suspension was mixed with 20 μg of plasmid DNA.

After 5 min at room temperature, cells were pulsed at 1000 μF and 250 V. After 10 min incubation at 37 °C, the suspension was diluted in medium and cultured for 24 h. Cells were replaced with fresh medium and treated with high salt (100 mM NaCl; 200 mosmol/kgH_2O) to the normal medium resulting in final osmolarity of 500 mosmol/kgH_2O or pretreated with specific inhibitors for 30 min before treatment of high salt for 18 h. After treatment, the cells were harvested and lysed with reporter lysis buffer (Promega Luciferase Assay system). The cell extract was mixed with the luciferase assay reagent and analyzed by the luminometer (Lumat LB 9507, EG&G Berthold, Bad Widbad, Germany).

2.3. Reverse transcription-PCR

Total RNA was extracted using Trizol Reagent according to the manufacturer's instruction. RNA pellets were dissolved in diethylpyrocarbonate-treated water. The yield of RNA was quantified by spectroscopy at 260 nm. Samples were aliquoted and stored at −80 °C until further processing. To synthesize first strand cDNA, 3 μg total RNA was incubated at 70 °C for 5 min with 0.5 μg of random hexamer and deionized water (up to 11 μl). The reverse transcription (RT) reaction was performed using 40 U of M-MLV reverse transcriptase (Promega, Madison, WI, USA) in 5 × reaction buffer (250 mmol/l Tris–HCl; pH 8.3, 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT), RNase inhibitor at 1 U/μl, and 1 mM dNTP mixtures at 37 °C for 60 min. The reaction was terminated by heating at 70 °C for 10 min, followed by cooling at 4 °C. The resulting cDNA was added to the PCR mixture containing 10 × PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 25 U of rTaq polymerase (TakaRa, Shiga, Japan), 4 μl of 2.5 mM dNTP mixtures, and 10 pmol of primers each. The final volume was 50 μl. Samples were amplified at 94 °C for 5 min, 23 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s using Mastercycler gradient (Eppendorf, Hamburg, Germany). β-actin was amplified for 20 cycles, followed by 72 °C for 5 min. The primers used were: COX-2 sense primer, 5′-TTCAAATGAGATTGTGGGAAAATTGCT-3′; COX-2 antisense primer, 5′-AGATCATCTCTGCCTGAGTATCTTT-3′[18]; β-actin sense primer, 5′-CCTGACCCTGAAGTACCCCA-3′, β-actin antisense primer, 5′-CGTCATGCAGCTCATAGCTC-3′; IL-8 sense primer, 5′-AAGGAACCATCTCACTG-3′, IL-8 antisense primer, 5′-GATTCTTGGATACCACAGAG-3′. The expected size of amplicons for COX-2, ®-actin, and IL-8 are 305, 550, 500, and 369 bp, respectively.

2.4. Western blot analysis

Protein extracted from A549 cells was isolated in lysis buffer (150 mM NaCl, 50 mM Tris–HCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% deoxycholate, 1% SDS) with protease inhibitor cocktail (Sigma) on ice for 1 h and then centrifuged for 20 min at 13000 × *g*. Supernatant was collected and protein concentrations were measured using the Bradford method (Bio-Rad). Proteins were dissolved in sample buffer and boiled for 5 min prior to loading onto an acrylamide gel. After SDS–PAGE, proteins were transferred to a polyvinylidene difluoride membrane, blocked with 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 60 min at room temperature. The membranes were incubated for 2 h at room temperature with 1:1000 dilution of COX-2 polyclonal antibody (Cayman, Ann Arbor, MI, USA). Equal lane loading was assessed using β-actin monoclonal antibody (Sigma). After washing with TBST, blots were incubated with 1:5000 dilution of the horseradish peroxidase conjugated-secondary antibody (Zymed, San Francisco, CA, USA), and washed again three times with TBST. The transferred proteins were visualized with an enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech, Buckinghamshire, UK).

2.5. Statistical analysis

Data were expressed as means ± S.E.M., and statistical analysis for single comparison was performed using the Student's *t* test. The criterion for statistical significance was $P < 0.05$.

3. Results

3.1. Hypertonic NaCl induces COX-2 mRNA and protein expression in A549 cells

We determined the effects of hypertonic NaCl on COX-2 expression in A549 cells by using RT-PCR and Western blot analyses. A549 cells were treated with 100 mM NaCl, 200 mM urea, or 200 mM mannitol to the normal medium resulting in final osmolarity of 500 mosmol/kgH_2O. As shown in Fig. 1A, membrane-permeable urea did not affect the COX-2 protein levels, but the membrane-impermeable agents, NaCl and mannitol, increased COX-2 protein levels in the A549 cells. This observation indicates that the induction of COX-2 regulation in A549 cells in the presence of a high salt concentration occurs in response to cell volume changes by tonicity rather than by the osmolarity of the surrounding fluid. The A549 cells were treated with hypertonic NaCl for 0, 0.5, 1, 2, 4, 6, or 18 h. COX-2 mRNA was not detected after 0 or 0.5 h of the high salt treatment, but COX-2 mRNA was detected after 1 h and continued to accumulate until 18 h. To examine whether the induction of COX-2 mRNA was the result of increased transcription from the COX-2 promoter, we performed a reporter assay using a luciferase construct containing the full-length mouse COX-2 promoter. As shown in Fig. 1C, hypertonic NaCl significantly increased the luciferase activity of the full-length COX-2 promoter in the A549 cells.

We examined whether the hypertonic NaCl induction of COX-2 gene expression requires protein synthesis by pre-treating A549 cells for 2 h with or without cycloheximide (10 μg/ml), a protein synthesis inhibitor, and then incubating the cells in the presence or absence of 100 mM NaCl for 18 h. As a positive control, we performed RT-PCR for interleukin-8 (IL-8) [19]. The increased expression of COX-2 mRNA induced by the high-salt medium was not affected by cycloheximide pretreatment (Fig. 1D), although cycloheximide alone significantly increased IL-8 mRNA in the A549 cells. This result suggests that protein synthesis is not involved and indicates that the response is elicited by pre-existing transcription factor(s), possibly by NF-κB.

3.2. Hypertonic NaCl regulation of COX-2 is not mediated by NF-κB in A549 cells

The transcription factor NF-κB is important in the hypertonic NaCl regulation of COX-2 in kidney cells [9]. The binding of activated NF-κB to the COX-2 promoter region is critical for COX-2 transcriptional activation in a number of cell types [20–22]. NF-κB is a positive regulator of COX-2 expression in macrophages and colon carcinoma cell lines

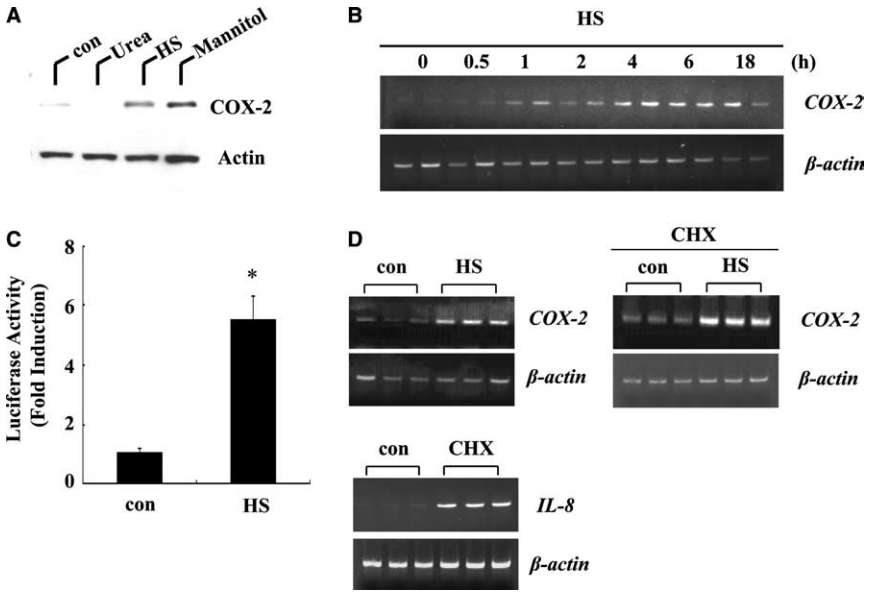

Fig. 1. Hypertonic NaCl increases COX-2 mRNA and protein expression in A549 cells. (A) A549 cells were treated with the indicated solutes (Urea, 200 mM urea; HS, 100 mM NaCl; mannitol, 200 mM mannitol) to the normal medium for 18 h. After incubation, the cell lysates were subjected to 10% SDS–PAGE and transferred to polyvinylidene difluoride membrane. Immunoblots were probed with a COX-2 antibody and reprobed with actin antibody. Bands were visualized by an ECL method, as described in Section 2. The immunoblot is representative of three independent experiments eliciting similar pattern. (B) A549 cells were treated with high salt (100 mM NaCl) for indicated time periods. Total RNA from A549 cells were analyzed for COX-2 mRNA expression by RT-PCR using specific primers as described in Section 2. Data presented are representative of two independent experiments showing similar trend. (C) A549 cells were transfected with COX-2-Luc and treated as indicated. After treatment, luciferase expression was determined as described in Section 2. Values represent the means ± S.E.M. ($N = 3$). *Represents $P < 0.05$. (D) A549 cells were pretreated with cycloheximide (10 μg/ml) for 2 h before incubation with 100 mM NaCl for 18 h. Total RNA from A549 cells were analyzed for COX-2 and IL-8 mRNA expression by RT-PCR assays. Data presented are representative of two independent experiments showing similar trend. con, untreated cells; HS, 100 mM NaCl; CHX, cycloheximide; IL-8, interleukin-8.

[23,24]. Studies have shown that IL-1β or lipopolysaccharide induces COX-2 expression via NF-κB activation in many cells including A549 cells [25–28]. To determine if NF-κB is involved in the hypertonic NaCl-induced COX-2 expression in A549 cells, we evaluated the effects of NF-κB inhibitors. We pretreated A549 cells with 5 μM MG132, a proteasome inhibitor that has been shown to prevent IκB degradation and thereby NF-κB activation [29], or 100 μM PDTC, followed by incubation in hypertonic NaCl for 18 h. As shown in Fig. 2A–C, neither MG132 nor PDTC inhibited COX-2 expression or promoter activation in cells exposed to hypertonic NaCl. No reduction in hypertonic NaCl induced COX-2 expression with MG132 or PDTC suggests that COX-2 activation occurs in the absence of NF-κB activation. Interestingly, we have consistently observed increased COX-2 protein expression with MG132 as compared with that of high salt (Fig. 2A, lanes 2 and 3). Similar results were observed in M-1 mouse cortical collecting duct cell (our unpublished results). We do not exactly understand how MG132 synergistically activates COX-2 in our model system. MG132 may activate upstream targets of hypertonic COX-2 activation by triggering other signaling transduction pathways independent of protein degradation [30].

To further determine the importance of NF-κB activation, we performed reporter assay using luciferase construct driven by the 5′-flanking region of the COX-2 promoter (−327/+59) containing NF-κB binding site. The COX-2 promoter (−327/+59) showed less than 2-fold activation in response to 100 mM NaCl (Fig. 2D). The 0.3 kb COX-2 promoter fragment with mutation at the NF-κB site (−223/−214) also had a marginal effect on luciferase expression (Fig. 2D). As a positive control for KBM, we used TPA [15]. These data suggest that NF-κB binding element is not critical in COX-2 upregulation by high salt in A549 cells. Furthermore, the hypertonic NaCl did not affect NF-κB-dependent luciferase expression in A549 cells. As a positive control for NF-κB-dependent gene transcription, we used YC-1, an activator of soluble guanylate cyclase, which initiates IKKα/β and NF-κB activation [31] (Fig. 2E). These observations indicate that NF-κB is not activated by hypertonic NaCl and imply that, although both salt and cytokines induce COX-2 expression, different signaling mechanisms exist for the hypertonic NaCl and cytokine induction of COX-2.

3.3. The hypertonic NaCl regulation of COX-2 in A549 cells is mediated by MEK1/2 and p38 MAPK

Study has shown that MAPK family members play a role in COX-2 gene expression induced by a hypertonic medium, which is crucial for cell survival under hyperosmotic shock [10]. The MAPK p38 is an essential component of the hypertonic signaling response pathway in mammals and is a major regulator in COX-2 upregulation [32–35]. To study the involvement of the MAPK pathway in the hypertonic NaCl induction of COX-2 expression in A549 cells, we evaluated the effects of p38 and MEK1/2 inhibitors on COX-2 expression. We pretreated A549 cells with SB203580 (a p38 MAPK inhibitor) or U0126 (a MEK1/2 inhibitor) for 30 min and then co-treated the cells with hypertonic NaCl for 18 h. The high-salt induction COX-2 mRNA and protein expression was

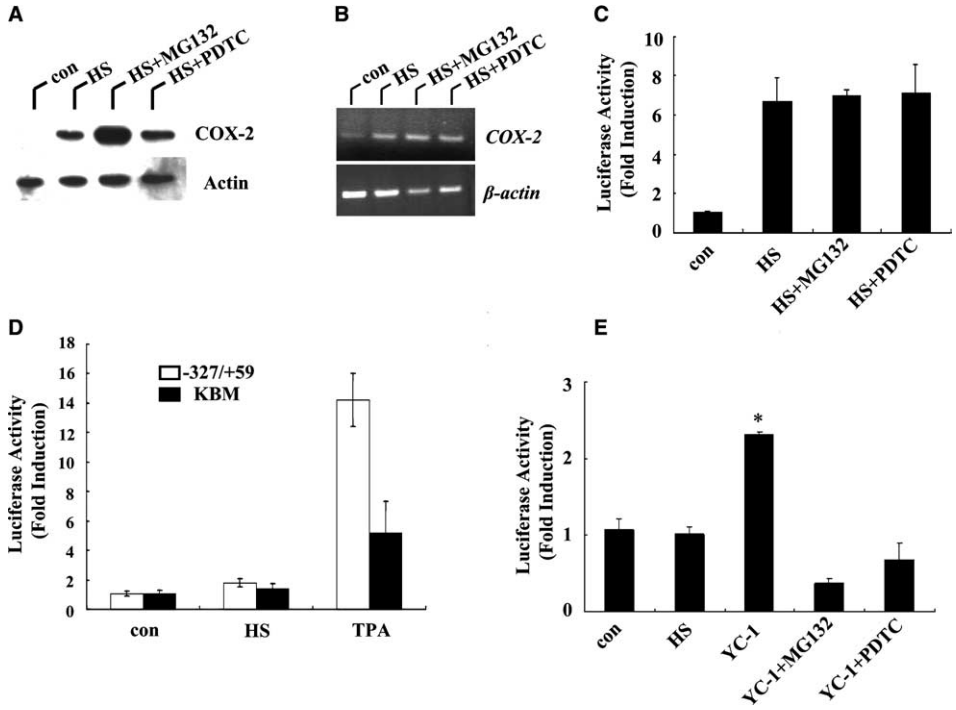

Fig. 2. NF-κB is not involved in hypertonic NaCl induction of COX-2 in A549 cells. (A) A549 cells were pretreated with PDTC (100 μM) or MG132 (5 μM) for 30 min before incubation with 100 mM NaCl for 18 h. Immunoblots were probed with a COX-2 antibody and reprobed with actin antibody, as described in Section 2. (B) A549 cells were pretreated with PDTC (100 μM) or MG132 (5 μM) for 30 min before incubation with 100 mM NaCl for 18 h. Total RNA from A549 cells were analyzed for COX-2 mRNA expression by RT-PCR assays, as described in Section 2. (C) A549 cells were transfected with COX-2-Luc and treated as indicated. After treatment, luciferase expression was determined as described in Section 2. Values represent the means ± S.E.M. ($N = 3$). (D) A549 cells were transfected with −327/+59 or KBM and treated as indicated. After treatment, luciferase expression was determined as described in Section 2. Values represent the means ± S.E.M. ($N = 3$). (E) A549 cells were transfected with the 3 × (NF-κB)tk-Luc and treated as indicated. After treatment, luciferase expression was determined. Values represent the means ± S.E.M. ($N = 3$). *Represents $P < 0.05$. con, untreated cells; HS, 100 mM NaCl. All experiments were repeated at least twice.

significantly blocked by SB203580 and U0126 (Fig. 3A and B). Furthermore, hypertonic NaCl-mediated full-length COX-2 promoter driven luciferase activity was partially inhibited by SB203580 and U0126 (Fig. 3C). It appears that p38 and MEK1/2 pathways affect not only at the level of transcription but also that of post-transcription in high-salt induction COX-2 expression in A549 cells. These results suggest that the activation of the p38 and MEK1/2 pathways is critical for the induction of COX-2 in A549 cells by a high-salt medium.

3.4. Dexamethasone inhibits hypertonic NaCl induction of COX-2 expression in A549 cells

Glucocorticoids regulate sodium uptake and fluid transport in both adult and fetal lungs, and studies have shown that a single dexamethasone injection increases AFC [36–40]. Dexamethasone suppresses COX-2 expression in the myelomonocytic leukemia cell line U937 [41]. The glucocorticoid receptor (GR) is involved in the tonic suppression of renal cortical COX-2 expression in animals [42]. To examine the possible

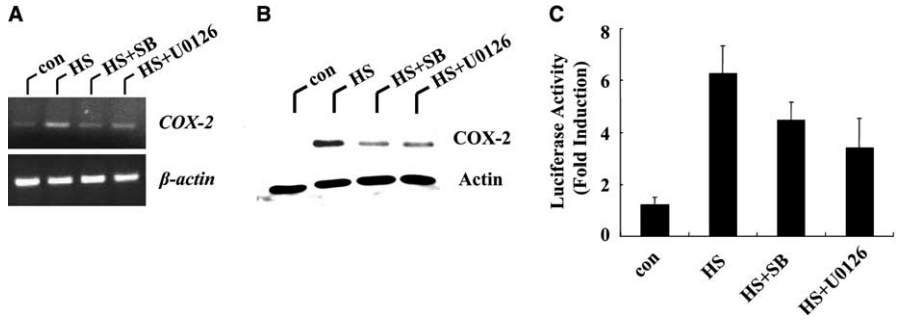

Fig. 3. MEK1/2 and p38 MAPK are involved in hypertonic NaCl induction of COX-2 in A549 cells. (A) A549 cells were pretreated with U1026 (10 μM) or SB203580 (10 μM) for 30 min before incubation with 100 mM NaCl for 18 h. Immunoblots were probed with a COX-2 antibody and reprobed with actin antibody, as described in Section 2. Data presented are representative of two independent experiments showing similar trend. (B) A549 cells were pretreated with U1026 (10 μM) or SB203580 (10 μM) for 30 min before incubation with 100 mM NaCl for 18 h. Total RNA from A549 cells were analyzed for COX-2 mRNA expression by RT-PCR assays, as described in Section 2. (C) A549 cells were transfected with COX-2-Luc and treated as indicated. After treatment, luciferase expression was determined as described in Section 2. Values represent the means ± S.E.M. ($N = 3$). con, untreated cells; HS, 100 mM NaCl; SB, SB203580. The immunoblot is representative of three independent experiments eliciting similar pattern.

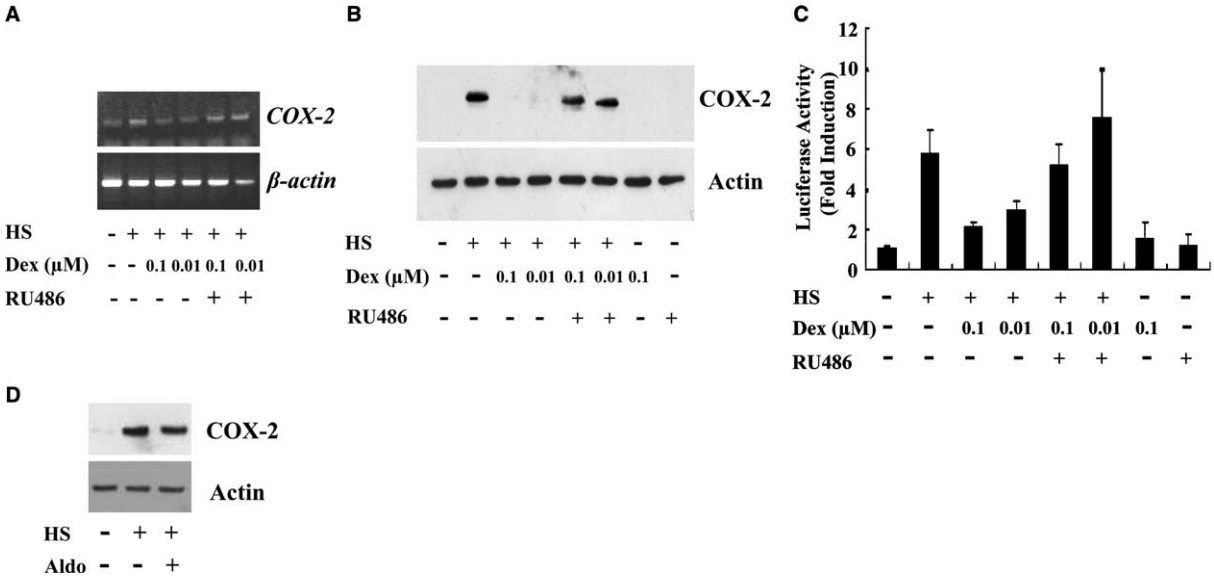

Fig. 4. Dexamethasone inhibits hypertonic NaCl induction of COX-2 in A549 cells. (A) A549 cells were pretreated with dexamethasone (0.01 or 0.1 μM) and/or RU486 (1 μM) for 1 h before incubation with 100 mM NaCl for 18 h. Total RNA from A549 cells were analyzed for COX-2 mRNA expression by RT-PCR assays, as described in Section 2. Data are representative of two independent experiments. (B) A549 cells were pretreated with dexamethasone (0.01 or 0.1 μM) and/or RU486 (1 μM) for 1 h before incubation with 100 mM NaCl for 18 h. Immunoblots were probed with a COX-2 antibody and reprobed with actin antibody, as described in Section 2. Blots are representative of four independent experiments showing similar pattern. (C) A549 cells were transfected with COX-2-Luc and treated as indicated. After treatment, luciferase expression was determined as described in Section 2. Experiments were repeated four times. (D) A549 cells were pretreated with aldosterone (0.1 μM) for 1 h before incubation with 100 mM NaCl for 18 h. Immunoblots were probed with a COX-2 antibody and reprobed with actin antibody. Blots are representative of two independent experiments showing similar pattern. Values represent the means ± S.E.M. ($N = 3$). *Represents $P < 0.05$. con, untreated cells; HS, 100 mM NaCl; Dex, dexamethasone; Aldo, aldosterone. Experiments were repeated twice.

role of the GR in the hypertonic NaCl induction of COX-2 in A549 cells, we pre-incubated cells with dexamethasone and/or the GR antagonist RU486 for 1 h and co-treated with NaCl. Neither dexamethasone nor RU486 in the absence of high salt had an effect on the COX-2 protein level or promoter activity. However, dexamethasone did block the hypertonic NaCl-induced COX-2 mRNA (Fig. 4A) and protein (Fig. 4B) expression, and COX-2 promoter activity (Fig. 4C). The suppressive effect of dexamethasone was antagonized by the GR antagonist RU486, suggesting that the response is mediated by the GR and glucocorticoid-specific. To further determine the specificity of the inhibitory effect of dexamethasone on salt-induced COX-2 regulation, we examined whether aldosterone exhibits similar suppressive effects. Cells were pre-incubated with aldosterone and/or the mineralocorticoid receptor antagonist spironolactone for 1 h and were co-treated with NaCl for 18 h. As shown in Fig. 4D, the application of aldosterone did not have an effect on the hypertonic NaCl induction of COX-2 protein expression in A549 cells. As a positive control, we performed an αENaC promoter-driven reporter gene assay; aldosterone significantly increased αENaC-dependent reporter gene transcription, and spironolactone blocked this induction (data not shown). These results indicate that dexamethasone inhibits hypertonic NaCl-induced COX-2 regulation through the GR in A549 cells.

4. Discussion

We studied the induction of COX-2 by hypertonic NaCl in lung epithelial A549 cells and observed that NaCl increases COX-2 expression at the level of transcription. Our study suggests that p38 and MEK1/2, and not NF-κB, are involved in the signal transduction leading to the expression of COX-2 induced by hypertonic NaCl. The NaCl induction of COX-2 in the lung cells could be a non-specific inflammatory-related response, but urea, a hyperosmotic agent that can promote cell lysis and can be irritating to cells, did not induce COX-2 in our study. COX-2 induction was observed in response to an increase in tonicity. The hypertonic NaCl induction of COX-2 has been studied most intensively in kidney tissue [6,7,9,10,43]. The expression of COX-2 in the kidney is tissue-specific; COX-2 is downregulated in the cortex and upregulated in the medulla [44]. Despite intensive in vivo and in vitro studies, the mechanism of the differential regulation in the kidney has not been elucidated. Our results show that hypertonic NaCl activates COX-2 expression in lung epithelial cells, as occurs in rat kidney medulla and inner medullary collecting duct cells [9,10,43].

The first candidate we investigated as a transcription factor responsible for COX-2 gene activation in lung epithelial cells was NF-κB. Studies have previously shown that hypertonic stress activates an NF-κB-COX-2-linked survival mechanism in renal medullary interstitial cells [43]. Other studies have shown that the inhibition of glycogen synthase kinase-3β protects renal cells from hypertonic stress via the induction of the NF-κB-COX-2-dependent pathway [9]. However, in contrast to the situation in the kidney medulla, the hypertonic activation of COX-2 in lung epithelial cells was not dependent on NF-κB. In addition, it appears that part of the signal transduction pathway leading to COX-2 activation is shared but is not identical with other stimuli, such as LPS and cytokines. The NaCl induction of COX-2 was inhibited by a glucocorticoid,

which agrees with the observations made in other studies of LPS and cytokines [45–47], while dexamethasone suppressed LPS- and IL-1β-induced COX-2 regulation in a NF-κB-dependent manner. Recent studies have shown that the inhibition of granulocyte-macrophage colony-stimulating factor by dexamethasone is independent of NF-κB [48,49], and the inhibition of NF-κB cannot account for all the repressive effects of dexamethasone on inflammatory genes such as COX-2 [50]. The transcription factor involved in the hypertonic NaCl activation of COX-2 remains to be identified, but it is most likely a pre-existing protein(s) rather than a newly synthesized protein, as shown by the cycloheximide experiments. It is possible that the factor is specific to the lung; however, we have observed the hypertonic NaCl activation of COX-2 in other cells, such as vascular smooth muscle (data not shown), which indicates that the response is not restricted to lung and kidney tissues. A few transcription factors activated by hypertonicity are known. One well-known example is tonicity-responsive enhancer (TonE) binding protein (TonEBP). TonEBP is a member of the Rel family of transcriptional activators that include NF-κB and nuclear factor of activated T cells [51]. Studies have previously shown that the hypertonic induction of COX-2 mRNA is not reduced by the expression of DN-TonEBP [52]. However, additional studies are needed to confirm that the hypertonic induction of COX-2 is independent of TonEBP. The identification of the transcription factor responsible for the hypertonic NaCl induction of COX-2 would greatly enhance our understanding of the hypertonicity-triggered signal transduction pathway that leads to COX-2 activation.

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